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PROSPECTS OF THE MASS CULTIVATION OF ALGAE AS BULK FODDER

(Perspektyvy masovoho vyroshchuvannya vodorostei dlya oderzhannya kormovoyi masy)

by

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In recent years one has witnessed, both in the Soviet Union and abroad, a wide development of investigations on the laboratory and industrial cultivation of unicellular algae. It is expected that they might be utilized in various fields of national economy. The increased interest in this object is explained to a large extent by its potentialities. In a number of cases (Sorokin, 1959) some strains of Chlorella double their biomass every three hours. Even in the case of a density of 1 g of dry substance of the algae per 1 litre of the substrate (in a layer of 10 cm in thickness) one can harvest within one year from 1 ha nearly 3000 tons of dry biomass. Since 1 litre of the nutrient medium will contain 10 g of cells -- in all 1% of the substrate weight -- 1 ha will yield during one year nearly 30,000 tons of organic substances. If it were possible to carry this into effect on a large scale, this would have meant a revolution in agriculture. That is why this problem deserves the greatest attention. Yet it seems to us that the theory and the practice of mass cultures of the algae proceeds from some fundamentally wrong principles which arose historically and inhibit at present the development and the great potentialities of this new trend.

At present algae are cultivated in mass cultures for the following national and scientific purposes:

1. As the initial material for the production of valuable preparations (for instance vitamins).

2. To obtain highly-active food additives, i.e. a biomass the introduction of which in a small proportion into the feed ration of animals results in a sharp increase of the animal growth. Such biomass contains a large quantity of biologically active substances.

3. To obtain fodder. In this case the proportion of algal biomass in the feed ration should amount to at least several per cent.

4. To obtain fertilizers. This includes the cultivation of the nitrogen-fixation algae for green manures and in the shape of the mother culture to be introduced on rice fields.

5. In the group of 'autographs' in the generation of ecological cycles in a closed space.

6. To bring into practice the biological purification of various living and industrial waste products.

Each of these tasks presents to a large extent an independent trend -- with its specific problems and ways of its realization. We shall analyse only the prospects and the possible ways of solving the problem of cultivating algae as the feed biomass for agricultural animals. For this end there are studied at present some representatives of the protococcus algae. The most favourite objects are the representatives of the genera Chlorella and Scenedesmus. Research into other algae is conducted much less frequently. Notwithstanding the diversity of methods and the large number of apparatus and equipment, the methods of mass cultivation of algae have some common elements. Here belong: (1) forced mixing of the liquid (bubbling, pumping, stirrers etc.); (2) supplementary supplying of cultures with carbon dioxide (other than from the air) from bottles, industrial waste gases etc.

As a rule one can cultivate algae also without stirring and without application of additional CO_2. However, this results in a sharp decrease of growth of the cultures used.

The cultivation of algae is not the end of the process of biomass production. Moreover, in a number of cases it is not even the most complex stage. The obtained culture must be isolated from the liquid and concentrated. There are known the following methods of separating cells from the medium: separation, agglutination with acid, application of coagulants, flotation, and usual settling. Each of these methods is entirely suitable under laboratory conditions. However, the application of these methods to the production of biomass in the semi-industrial cultivation of algae results already in a series of complications. And none of these methods is suitable for large-scale industrial production. When the thickness of the liquid layer is 10 cm it is necessary to treat 1000 m^3 of the liquid in order to harvest the crop from 1 ha. And since when the time of doubling of the cell number attains the theoretical value it will be necessary to pass through the cell-collecting systems 8000 m^3 of liquid per ha per 24 hours.

In each industry there exists a theoretical limit of capacity, which can be reached under specific conditions. In the mass cultures of the algae this limit depends on solar energy obtained by the algae. For the industrial conditions at which cultivation is conducted on thousands of ha, it is inexpedient to use artificial lighting at night.

When selecting rapidly-propagating strains and creating for them the optimum conditions, including the hotbed effect and the artificial heating in winter, one can assume that the quantity of assimilated energy attains 20%, i.e. a magnitude which, though not easily, has been still realized in practice.

During the optimum growth conditions the main limiting factor which determines the biomass increment is the light. Indeed, one can select a very rapidly growing strain, choose for it the best conditions, create the successful cultivator (sic) designs, but, in the case of the really mass cultivation, one will utilize virtually only the sunlight energy. Any artificial lighting will only contribute to the increase of production costs.

In the research by Stanko and others (1958) results are adduced for Kazakhstan -- a country highly favourable with respect to the amount of the incident solar energy. After having treated the experimental data adduced by the authors, we obtained the following values: for the altitude of 780 m above sea level there falls on the area of 1 ha during 24 hours 8.23×10^7 kcal of solar radiation in the visual spectrum range, and for the altitude of 2980 m -- 1.15×10^5 kcal per ha per 24 hours. Assuming that the amount of absorbed energy in terms of the incident one is 20%, that the number of photons with the wavelength of 5500 Å (i.e. a wavelength in the middle of the spectrum), which is consumed for the regeneration of one molecule of carbon dioxide, is 4, and having carried out some simplifications in calculations, we obtain the following magnitudes. At the altitude of 780 m above sea level the energy would suffice for obtaining an alga harvest, which would amount to 10,950 quintals/ha of dry substance per year, the corresponding figure for the altitude of 2980 m being 15,250 quintals/ha. In comparison with the crop capacity of many agricultural cultures these figures can be undoubtedly called record' ones, especially if one takes into account that the yields of cultivated plants are usually expressed in green weight. Nevertheless, the conversion to dry substance of the most fruitful cultures (such as maize for silage, sugar-beet etc.) will reduce the usual productivity number roughly by the factor of 4 - 5.

Under the mass-culture conditions one failed even to approach the above-mentioned theoretically possible productivity figures.

According to the data of foreign literature, the productivity of the blue-green alga Tolypothrix tenuis, when converted to 1 ha, amounted to 700 quintals per year (Watanaba et al., 1959). According to the data of Li Shang-hao et al. (1959), Scenedesmus obliquus produced a biomass of 750 quintals/ha per year. In the Soviet Union this problem was studied in most detail at the Biological Institute of Leningrad University. The maximum increment corresponded to the value which would reach 514 quintals/ha of biomass per year (Chesnokov et al., 1960). Higher crops can be obtained under laboratory conditions. Thus, Moshkov (1964) quotes the increment figure of 40 g of dry substance from 1 m^2 during 24 hours. This corresponds to 1460 quintals/ha per year. However, the complex nature of the apparatus and of the technological process are very high in this case.

We failed to find in literature and indications with respect to the cost price of the biomass of algae cultivated in mass cultivation. Therefore we shall adduce below a theoretical analysis of this cost price.

The most economical is the method of mixing liquids with the aid of mixers. A mixer driven by a 1-kW motor will hardly be able to effect a satisfactory mixing on an area in excess of 100 m^2. Hence at least 100 such mixers will be required for 1 ha. The theoretically possible annual increment of 15,250 quintals/ha will require the consumption of at least the same quantity o. carbon dioxide(by weight). Finally it will be indispensable to separate at least once in every 24 hours the culture liquid to isolate the rapidly growing biomass, i.e. 1000 m^3 from each hectare. One of the existing centrigugces, with a motor of 10 kW, ensures the separation of cells from 100 litres in the course of 1 hour. Let us assume that we shall succeed in increasing the productivity of the separators tenfoldand also to increase by the factor of 10 their characteristic per apparatus. Then the apparatus with a power of 100 kW will be able to treat 10 m^3 of the liquid in the course of 1 hour. Ten such apparatus will be required to effect the separation of the culture liquid at night. Consequently to produce 15,250 quintals of dry biomass it will be necessary to consume 4,8000000 kWh of electrical energy.yearly. If we assume that the depreciation of the equipment equals the value of the energy which passes through it, and if we add to this the commercial value of carbon dioxide, we shall find that 1 quintal of dry biomass will cost 350.20 roubles. And this figure does not include the cost of the building where the algae are cultivated, the wages of the service personnel, nor the transport and other costs.

For the sake of comparison let us remind that the commercial value of 1 quintal of potatoes is 9 roubles. The improvement of technical means will reduce the cost price of the biomass, but even in this case it will be higher than the cost price of ordinary feeds.

The fundamental criterion of the expediency of an industry is its profitableness. At present we may call profitable the mass cultures of algae which are grown for obtaining valuable products, e.g. carotene. Perhaps it is also profitable to produce a biomass which contains biologically active substances which are used as feed additives.

Besides the achievement of the method of concentration of the biomass, for instance, by the biological coagulation in air tanks of a certain type, there will be likewise profitable the culture of algae which propagate during the purification of sewage, since all expenses for the cultivation of algae are related here to the organization of the purification process of effluents, and the cost of the biomass equals virtually the expenses for the concentration of the biomass from the culture liquid.

A special introduction of the mass cultures of algae with a view to obtaining food biomass is, as follows from calculations, not yet profitable. Moreover, given the present principles of the cultivation of mass cultures, this is not likely to happen at all in the more or less near future. To render the production of feed on the base of the mass algal cultures profitable, it is indispensable to proceed from other principles of organization of the process of cultivation and culture selection.

The existing criteria of the mass cultivation of algae were formed on the basis of a randomly chosen object -- Chlorella. One transferred into industrial production the laboratory techniques of cultivation of this organism: forced mixing, artificial supplying with carbon dioxide, indispensability of a complicated system of the removal of biomass etc. This is explained by the fact that Chlorella had been studied for a long time as a convenient laboratory model. Its physiology and biochemistry are better known than for other algae. For this organism suitable compositions of nutrient media had been developed, and the conditions of cultivation were also well developed even prior to investigations into mass cultures. Finally, a good effect was obtained immediately the cultures were started, i.e. the algae did grow and did not die away. This was the cause of the "Chlorella hypnosis. The entire problem of the mass cultures of algae was viewed through the Chlorella prism, and the entire technology of cultivation was created started from the basic foundations of Chlorella cultivation. This alga became a standard of a kind. All main strains were compared with it, under conditions which had been specifically developed for Chlorella.

Consequently there were adopted as criteria for mass cultivation: no special claims to the medium, fast growth rate (from the data of laboratory investigations), high protein content, high competition with respect to the contaminating micro-organisms. Sometimes the culture requirements can be given in much more detail. Thus, Vladimirova and Semenenko (1962) indicate that, in order to be used in mass cultures, the algal strains should satisfy the following requirements: high photosynthesis intensity during cultivation on mineral media with a high concentration of salts; high growth energy when illuminated with high-intensity light; thermophilic properties; high resistance against the penetration of extraneous organisms and ability to suppress the growth of the choking microflora (bacteria and algae). It should be also mentioned that the most convenient for use in industrial cultures are the unicellular or small-colony forms of algae.

With respect to Chlorella the above-mentioned requirements are certainly expedient. However, under conditions approved for Chlorella, other species may grow badly, and the insufficient investigation of their physiology does not permit to elucidate the optimum conditions for their cultures; besides, the conditions which are best for Chlorella, are not best from the economic point of view. We think that the mass culture of algae can be only compared with cultivated plants, since the former can be justified only if they give a higher biomass yield than the latter do. No intermediate standard (of the Chlorella type) is required here.

In the case of cultivated plants there are no expenses related to the separation, mixing of the liquids, additional supplying with gaseous carbon dioxide etc. The criteria of the mass cultivation of algae should proceed from these facts. We think that the strains used in cultures, and the process itself of their cultivation should meet the following requirements.

1. The culture should be capable of a good growth without mixing. In the case of Chlorella this requirement is unacceptable. Mixing improves the gas exchange, the conditions of illumination, and prevents the settling of the culture. Settling worsens the growth of unicellular algae in nature, where in a number of cases the intensive mixing is lacking. However, many algae grow fairly intensively under these conditions. To tell the truth, Chlorella has a poor growth under such conditions. It is therefore necessary to look for the strains in other groups of algae. Proceeding from general considerations it appears that of promise may be the strains which grow as a film either on the surface of the liquid or under its thin layer on the bottom of the cultivation vessel.

2. The culture should grow without additional feeding with gaseous carbon dioxide. The complications related to additional feeding with carbon dioxide are of twofold nature. In the first place, the supply of gaseous carbon dioxide from cylinders is expensive. Secondly, a suitable plant in the vicinity is indispensable. The additional feeding itself complicates the process of cultivation.

In nature, the higher plants, and the algae, thrive on the ordinary atmospheric carbon dioxide. However, both kinds of plants react positively to the increase (within certain limits) of this gas in the atmosphere. Unlike the higher plants, some algae are able to assimilate carbon from solutions of carbonates. These compounds are much cheaper and easier transportable than carbon dioxide in gaseous state. Therefore, when choosing cultures for the mass cultivation of algae, one should give preference to those which, firstly, grow fairly well at the usual CO_2 content of air, and, secondly, utilize intensively the carbonate carbon.

3. The culture should grow without combined nitrogen nearly as well as in its presence. A sterile mass culture is virtually impossible. Moreover, it is also inexpedient from the point of view of economics. However, the entry of various micro-organisms into the cultivation vessels is not always without effect. According to our observations the cultures of algae suffer most frequently from algal contamination. Blue-green algae can develop in vessels with Chlorella, and vice versa. Such contamination results almost always in a sharp drop of the biomass crop.

The representatives of the genera Chlorella and Scenedesmus appear particularly frequently as the contaminating agent. Their interrelations and the final result depend to a large extent on the composition of the nutrient medium and on the conditions of cultivation. If one utilizes nitrogen-fixing algae and a nitrogen-free medium, the result of the struggle between the growing culture and the contaminating culture will nearly always turn out to the benefit of the former. Only if the contaminating culture is also a nitrogen-fixing one, does the state of affairs become complicated. However, the probability of this occurrence is immeasurably less than the possibility of contamination with nitrogen-non-fixing cultures. Besides, to be competitive with the main culture, the contaminating nitrogen-fixing agent ought to have a high growth rate -- and this is seldom the case.

In principle the mass culture can be conducted when the fixed-nitrogen content of the medium is low, and when contamination is detected one can stop completely the supply of nitrogen. If strains are used, which grow well without nitrgen salts (sic), this simplifies and cheapens even more the process of production of algal biomass.

4. The culture ought to be 'thermo-tolerant'. It is at present almost generally accepted that most promising are the thermophilic algae. Nevertheless it seems to us that this is not the case. We call thermophilic those micro-organisms which grow better at higher temperatures. In laboratory conditions the thermophilic organisms are really promising. However, in the mass cultivation of algae the higher temperature in the cultivating vessels is not likely to be maintained during a greater part of the day. This is tantamount to saying that the thermophilic organisms will not be able to develop at their optimum temperature. Also the [illegible] mesophils / will find themselves in a disadvantageous position. In the best conditions will be algae with an extended temperature optimum, which do not fear a temperature rise and do not stop their growth in this case.

5. The separation of biomass should be carried out with simple and economically advantageous methods. At first sight this is a merely technical problem. We think nevertheless that it completely depends on the organism chosen and on its growth characteristic. For Chlorella it is difficult to expect a simple solution of this problem. But when the cells of the algae will form large aggregates, which settle rapidly, settling itself will make it possible

to concentrate the biomass to such an extent as to separate it subsequently. Even still more simply and economically more advantageously one can collect with scrapers the culture crop that grows as a film on the bottom of the building. Finally, when the algae will grow as a film on the surface of the liquid, it will be possible to collect them with rakes.

6. The culture must not be toxic. To be used as feed the biomass must not be toxic, or at least its toxicity should be removable by such uncomplicated methods as drying or short preparation.

7. The culture liquid should be utilized. The algae secrete into the medium a considerable quantity of organic substances. Therefore the problem of utilizing these substances is very urgent. This liquid can be fed to animals. However this is practicable when the culture contains a relatively small quantity of liquid. A very high biomass concentration is not compulsory. Thus, for some blue-green algae the dry-matter content of the green biomass is less than 4%. Consequently, when the density of the dry matter is 10 g/l the cells will contain not less than a quarter of the entire liquid. It is possible in principle to obtain even a considerably higher density. Consequently the quantity of the supernatant liquid will be comparatively small.

To put these principles into practice it is indispensable to change radically the practice of culture selection. Up till now the work used to begin with the utilization of the strain already available in collections. Instead, it is necessary to begin with the isolation of strains selected in nature, which would meet the established requirements. The initially selected cultures, just as the cultivated plants, must undergo a selection. Next it is necessary to study the physiology of nutrition of the obtained variants, and only then to check them in mass culture. It seems to us that only then is it possible to expect a progress in the mass cultivation of algae with a view to obtaining a biomass to be used as feed.

A question may arise -- are the enumerated requirements not too impracticable? According to previous results the answer is -- no. At the present time cultures were obtained at the Institute of Microbiology and Virusology of the Academy of Sciences of the Ukr.S.S.R., which grow on the surface of water and can be therefore easily separated from the medium, which assimilate the carbon from carbonates, do not require mixing and grow well as the expense of atmospheric nitrogen, and finally are resistant to temperatures up to 40°C.

It should be hoped that further research, based on the above-mentioned criteria, will result in strains which are even better than those obtained till now.

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